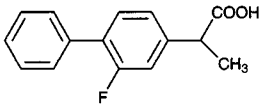
**OTHER SUBSTANCES**
**Extracted:** morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone
**Interfering:** imipramine, amitriptyline

**KEY WORDS**
column-switching

**REFERENCE**
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

# Flurbiprofen




**Molecular formula:** $C_{15}H_{13}FO_2$
**Molecular weight:** 244.27
**CAS Registry No.:** 5104-49-4
**Merck Index:** 4234
**Lednicer No.:** 1 86

**SAMPLE**
**Matrix:** aqueous humor
**Sample preparation:** 100 μL Aqueous humor + 500 μL MeCN + 30 μL 400 ng/mL (+)-naproxen in MeOH, mix mechanically for 90 s, centrifuge at 3000 g for 20 min. Remove the supernatant and dry it under nitrogen at room temperature, dissolve the residue in 50 μL mobile phase by swirl-mixing for 1 min, centrifuge at 3000 g for 20 s, reduce volume to 20-30 μL, inject an aliquot.

**HPLC VARIABLES**
**Column:** 150 × 4.5 5 μm Ultrasphere octyl
**Mobile phase:** MeCN:triethylamine:1.65% glacial acetic acid 505:0.65:495, pH 4.35
**Column temperature:** 30
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 280

**CHROMATOGRAM**
**Retention time:** 6.04
**Internal standard:** naproxen (3.89)
**Limit of detection:** 0.4 ng

**OTHER SUBSTANCES**
**Extracted:** diclofenac, indomethacin, meclofenamic acid
**Simultaneous:** bacitracin, cortisone acetate, diazepam, fluorometholone, hydrocortisone acetate, imipramine, ketoprofen, ketorolac tromethamine, levobunolol, metipranolol, neomycin, prednisolone acetate, proparacaine, propranolol, salicylic acid, sulfacetamide, suprofen
**Noninterfering:** acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide

**KEY WORDS**
human; rabbit

### REFERENCE

Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, *654*, 140–145.

### SAMPLE

**Matrix:** aqueous humor

**Sample preparation:** 100 μL Aqueous humor + 500 μL MeCN + 30 μL 400 ng/mL (+)-naproxen, mix mechanically for 90 s, centrifuge at 3000 g for 20 min. Remove supernatant and dry it under nitrogen at room temperature. Dissolve residue in 50 μL mobile phase by swirl mixing for 1 min, centrifuge at 3000 g for 20 s. For concentrations of < 20 ng/mL, reduce volume to 20-30 μL under nitrogen.

### HPLC VARIABLES

**Column:** 150 × 4.5 5 μm Ultrasphere octyl

**Mobile phase:** 505 mL MeCN containing 0.65 mL triethylamine + 495 mL 1.65% glacial acetic acid, apparent pH 4.35

**Column temperature:** 30

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 280

### CHROMATOGRAM

**Retention time:** 6.04

**Internal standard:** naproxen (3.89)

**Limit of detection:** 4 ng/mL

### OTHER SUBSTANCES

**Extracted:** diclofenac

**Simultaneous:** bacitracin, cortisone, diazepam, fluorometholone, hydrocortisone, imipramine, indomethacin, ketoprofen, ketorolac, levobunolol, meclofenamic acid, metipranolol, neomycin, prednisolone, proraracaine, propranolol, salicylic acid, sulfacetamide, suprofen

**Noninterfering:** acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide.

### KEY WORDS

rabbit; human

### REFERENCE

Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, *654*, 140–145.

### SAMPLE

**Matrix:** blood

**Sample preparation:** Add 125 μL MeCN to 50 μL plasma, mix, centrifuge at 9000g for 10 min, inject a 10 μL aliquot of the supernatant.

### HPLC VARIABLES

**Guard column:** 5 μm C18 (Brownlee)

**Column:** 250 × 4.6 5 μm RP C18 (Hibar, Merck, Germany)

**Mobile phase:** MeCN:water:phosphoric acid 60:40:0.05

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** F ex 250 em 285

### CHROMATOGRAM

**Retention time:** 3.4

**Limit of detection:** 50 ng/mL

### KEY WORDS

rat; plasma; pharmacokinetics

**REFERENCE**
Park,K.-M.; Gao,Z.-G.; Kim,C.-K. Assay of flurbiprofen in rat plasma using HPLC with fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1849–1855.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Mix 500 μL plasma with 50 μL 200 μg/mL IS in MeOH. Add 500 μL 1 M HCl, extract with 10 mL dichloromethane. Separate the organic layer and evaporate it under a stream of nitrogen at 30°. Reconstitute the residue with 500 μL mobile phase. Inject a 50 μL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 3.9 10 μm C18 μBondapak
**Mobile phase:** MeCN:MeOH:1% pH 5.8 glacial acetic acid 19:19:62
**Flow rate:** 1.8
**Injection volume:** 50
**Detector:** UV 260

**CHROMATOGRAM**
**Retention time:** 5.65
**Internal standard:** 2-(2'-chloro-4-biphenyl)propionic acid (7.99)
**Limit of detection:** 500 ng/mL

**KEY WORDS**
plasma

**REFERENCE**
Pargal,A.; Kelkar,M.G.; Nayak,P.J. The effect of food on the bioavailability of ibuprofen and flurbiprofen from sustained release formulations, *Biopharm.Drug Dispos.*, **1996**, *17*, 511–519.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 500 μL Plasma + 1 mL water + ibuprofen (15 μg per 100 μL) + 2 mL 10% trichloroacetic acid, mix, add 5 mL hexane, mix for 15 min, centrifuge at 800 g for 5-10 min, repeat extraction. Combine hexane layers and evaporate them under a stream of nitrogen at 37-40°. Reconstitute with 300 μL chloroform, add 200 μL 65 mg/mL 1,1'-carbonyldiimidazole in chloroform, let stand 5-10 min at room temperature, add 10 μL glacial acetic acid, vortex briefly, let stand 5-10 min at room temperature, add 50 μL S-(α)-methylbenzylamine, mix briefly, let stand for 30 min at room temperature, add 3 mL 0.5 M ammonium hydroxide, add 5 mL hexane, mix gently for 15 min. Remove hexane and wash it with 3 mL 1 M HCl, 3 mL 0.5 M ammonium hydroxide, and 3 mL 1 M HCl (with 15 min mixing each time). Evaporate hexane under a stream of nitrogen at 37°, dissolve in 150 μL mobile phase, inject 25 μL aliquot.

**HPLC VARIABLES**
**Guard column:** Brownlee RP18
**Column:** 250 × 4.6 5 μm Ultrasphere ODS
**Mobile phase:** MeCN:water 62:38
**Flow rate:** 1
**Injection volume:** 25
**Detector:** UV 245

**CHROMATOGRAM**
**Retention time:** 12 (S), 14 (R)
**Internal standard:** ibuprofen (17 S, 19 R)
**Limit of detection:** 10 ng/mL
**Limit of quantitation:** 25 ng/mL

**OTHER SUBSTANCES**
**Simultaneous:** metabolites

**KEY WORDS**
plasma; derivatization; chiral

**REFERENCE**

Knadler,M.P.; Hall,S.D. High-performance liquid chromatographic analysis of the enantiomers of flurbiprofen and its metabolites in plasma and urine, *J.Chromatogr.*, **1989**, *494*, 173–182.

**SAMPLE**

**Matrix:** blood

**Sample preparation:** 500 μL Plasma + 1.25 μg S-Naproxen + 200 μL 2 M HCl + 6 mL hexane: diethyl ether 8:2 (ice cold), extract, centrifuge at 1500 g for 5 min. Remove 5 mL of organic layer and evaporate it to dryness under a stream of nitrogen, redissolve in 500 μL 30 mM pH 7.5 phosphate buffer, inject 50-100 μL aliquot

**HPLC VARIABLES**

**Column:** 100 × 4 5 μm Grom AGP

**Mobile phase:** 2-Propanol:20 mM pH 6.5 phosphate buffer 5:95 containing 1 mM dimethyloctylamine

**Column temperature:** 15

**Flow rate:** 0.8

**Injection volume:** 50-100

**Detector:** UV 246

**CHROMATOGRAM**

**Retention time:** 7.6 (R), 15.2 (S)

**Internal standard:** S-naproxen (5.2)

**Limit of quantitation:** 50 ng/mL

**KEY WORDS**

plasma; chiral

**REFERENCE**

Geisslinger,G.; Menzel-Soglowek,S.; Schuster,O.; Brune,K. Stereoselective high-performance liquid chromatographic determination of flurbiprofen in human plasma, *J.Chromatogr.*, **1992**, *573*, 163–167.

**SAMPLE**

**Matrix:** blood

**Sample preparation:** 500 μL Plasma + 150 μL 1 M phosphoric acid + 5 mL hexane:ether 80: 20, extract. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

**HPLC VARIABLES**

**Column:** 4 μm Nova Pak C18

**Mobile phase:** MeCN:water:acetic acid 59:40.5:0.5

**Flow rate:** 1.3

**Detector:** UV 233

**CHROMATOGRAM**

**Internal standard:** flurbiprofen

**OTHER SUBSTANCES**

**Extracted:** ibuprofen

**KEY WORDS**

plasma; flurbiprofen is IS

**REFERENCE**

al-Meshal,M.A.; El-Sayed,Y.M.; al-Balla,S.R.; Gouda,M.W. The effect of colestipol and cholestyramine on ibuprofen bioavailability in man, *Biopharm.Drug Dispos.*, **1994**, *15*, 463–471.

**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 100 μL 1 M HCl + 100 μL water + 6 mL ether:hexane 20:80, shake for 10 min, centrifuge at 900 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 100 μL 10 mM NaOH, sonicate for 3 min, add 50 μL 100 mM pH 7.0 phosphate buffer containing 0.1% dimethyloctylamine, sonicate for 3 min, inject a 5 μL aliquot.

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**HPLC VARIABLES**
**Guard column:** 10 × 3 5 μm Chiral-AGP (ChromTech)
**Column:** 100 × 4 5 μm Chiral-AGP (ChromTech)
**Mobile phase:** Gradient. A was 10 mM pH 7.0 phosphate buffer containing 1 mM dimethyloctylamine. B was isopropanol:10 mM pH 7.0 phosphate buffer 50:50 containing 1 mM dimethyloctylamine. A:B 99.2:0.8 for 5 min, to 59:41 over 10 min, re-equilibrate for 10 min.
**Flow rate:** 0.9
**Injection volume:** 5
**Detector:** UV 220 for 7 min, then UV 245

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**CHROMATOGRAM**
**Retention time:** 9.5, 11.6 (enantiomers)
**Internal standard:** flurbiprofen

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**OTHER SUBSTANCES**
**Extracted:** ibuprofen

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**KEY WORDS**
plasma; chiral; flurbiprofen is IS

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**REFERENCE**
de Vries,J.X.; Schmitz-Kummer,E.; Siemon,D. The analysis of ibuprofen enantiomers in human plasma by high-performance liquid chromatography on an α1-acid glycoprotein chiral stationary phase, *J.Liq.Chromatogr.*, **1994**, *17*, 2127–2145.

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**SAMPLE**
**Matrix:** blood
**Sample preparation:** 100 μL Plasma + + 25 μL 2 M HCl, vortex for 15 s, add 2 mL isooctane:isopropanol 85:15, rotate for 5 min, centrifuge at 3000 rpm for 10 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 25 μL 5 mg/mL 5-bromoacetyl acenaphthene in MeCN, add 10 μL 3% triethylamine in MeCN, vortex for 30 s, heat at 75° for 5 min, evaporate to dryness under reduced pressure, reconstitute with 25 μL MeCN, inject a 20 μL aliquot. (Prepare 5-bromoacetyl acenaphthene as follows. Add 43 g bromoacetyl chloride to 43 g acenaphthene dissolved in 200 mL dichloroethane, cool to -5° in an ice/salt bath, stir vigorously and add 38 g aluminum chloride in small portions over 90 min, do not allow temperature to go above 3°, place under reduced pressure for 30 min, add an excess of crushed ice. Separate the dichloroethane layer and wash it with two 100 mL portions of dilute HCl, wash with 100 mL 5% sodium carbonate solution. Dry the organic layer over anhydrous magnesium sulfate, remove the solvent under reduced pressure, allow the oily residue to solidify, remove liquid by blotting with filter paper. Purify the solid by chromatography on a 300 × 20 column of 60-120 mesh silica gel, elute with toluene, unreacted acenaphthene elutes first followed by 5-bromoacetyl acenaphthene (mp 87-90°).)

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**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Hypersil C18
**Mobile phase:** MeCN:water 90:10
**Flow rate:** 1
**Injection volume:** 20
**Detector:** F ex 250 em 450

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**CHROMATOGRAM**
**Internal standard:** flurbiprofen

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**OTHER SUBSTANCES**
**Extracted:** ibuprofen

### KEY WORDS
rat; plasma; protect from light; derivatization; flurbiprofen is IS

### REFERENCE
Gifford,L.A.; Owusu-Daaku,F.T.K.; Stevens,A.J. Acenaphthene fluorescence derivatization reagents for use in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *715*, 201–212.

### SAMPLE
**Matrix:** blood
**Sample preparation:** 500 μL Plasma + 125 μL 40 mM decanoic acid in MeCN, mix. Dialyze a 100 μL sample against 20 mM pH 7.0 phosphate buffer using a Gilson Cuprophane membrane (molecular mass cut-off 15 kDa). Continuously pump the buffer through the dialysis cell and through column A at 3 mL/min for 9.6 min, backflush the contents of column A onto column B with the mobile phase, monitor the effluent from column B. (After each injection flush plasma channel with 1 mL 0.05% Triton X-100, with 1 mL 1 mM HCl, and with 2 mL water. After each injection flush buffer channel with 3 mL 20 mM pH 7.0 phosphate buffer and condition column A with 1 mL 20 mM pH 7.0 phosphate buffer.)

### HPLC VARIABLES
**Column:** A 10 × 2 40 μm Bondesil C18 (Analytichem); B 250 × 3.1 5 μm C18 (RoSil Research Separation Laboratories)
**Mobile phase:** MeCN:MeOH:20 mM pH 3.2 phosphate buffer 50:10:40
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 247

### CHROMATOGRAM
**Limit of detection:** 10 ng/mL

### OTHER SUBSTANCES
**Extracted:** fenoprofen (UV 272), ibuprofen (UV 264), ketoprofen (UV 261), naproxen (UV 272)

### KEY WORDS
plasma; dialysis; column-switching

### REFERENCE
Herráez-Hernández,R.; Van de Merbel,N.C.; Brinkman,U.A.T. Determination of the total concentration of highly protein-bound drugs in plasma by on-line dialysis and column liquid chromatography: application to non-steroidal anti-inflammatory drugs, *J.Chromatogr.B*, **1995**, *666*, 127–137.

### SAMPLE
**Matrix:** blood
**Sample preparation:** 500 μL Plasma + 100 μL 25 μg/mL indomethacin + 500 μL 600 mM sulfuric acid + 15 mL dichloromethane, mix for 20 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL 50 mM triethylamine in MeCN + 50 μL 60 mM ethyl chloroformate in MeCN, vortex for 30 s, add 50 μL 100 mM L-leucinamide in MeOH:triethylamine 100:14, let stand for 2 min, add 50 μL water, inject a 10-50 μL aliquot of the reaction mixture.

### HPLC VARIABLES
**Column:** 250 × 4.6 5 μm Ultrabase C18 (Shandon)
**Mobile phase:** MeCN:60 mM $KH_2PO_4$:triethylamine 49:51:0.1
**Flow rate:** 1.8
**Injection volume:** 10-50
**Detector:** UV 275

### CHROMATOGRAM
**Retention time:** 3.5 (R-(-)), 4.4 (S-(+))
**Internal standard:** indomethacin (5.3)
**Limit of detection:** 100 ng/mL

**OTHER SUBSTANCES**
**Extracted:** ibuprofen (UV 225), ketoprofen

**KEY WORDS**
plasma; chiral; derivatization

**REFERENCE**
Péhourcq,F.; Lagrange,F.; Labat,L.; Bannwarth,B. Simultaneous measurement of flurbiprofen, ibuprofen, and ketoprofen enantiomer concentrations in plasma using L-leucinamide as the chiral coupling component, *J.Liq.Chromatogr.*, **1995**, *18*, 3969–3979.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

**HPLC VARIABLES**
**Column:** 300 × 3.9 4 μm NovaPack C18
**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
**Column temperature:** 30
**Flow rate:** 0.8
**Injection volume:** 50
**Detector:** UV 247

**CHROMATOGRAM**
**Retention time:** 8.01
**Limit of detection:** <120 ng/mL

**KEY WORDS**
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidra-

mine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

### REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

### SAMPLE

**Matrix:** blood, milk

**Sample preparation:** 200 μL Plasma or milk + 20 μL 30% perchloric acid + 200 μL 2 μg/mL IS in MeOH, vortex for 2 min, add 20 μL 5 M NaOH, centrifuge at 5000 g. Remove a 200 μL aliquot of supernatant and add it to 200 μL mobile phase, mix, centrifuge, inject a 50 μL aliquot.

### HPLC VARIABLES

**Guard column:** Waters C18 Guard Pak
**Column:** 100 × 8 10 μm μBondapak C8 Radial Pak
**Mobile phase:** MeCN:MeOH:1% acetic acid 30:30:40
**Flow rate:** 2
**Injection volume:** 50
**Detector:** UV 254

### CHROMATOGRAM

**Retention time:** 6
**Internal standard:** 2-(2'-chloro-4-biphenylyl)propionic acid (7)
**Limit of detection:** 50 ng/mL

### KEY WORDS

plasma

### REFERENCE

Johnson,V.A.; Wilson,J.T. Flurbiprofen analysis in plasma and breast milk by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *382*, 367–371.

### SAMPLE

**Matrix:** blood, synovial fluid

**Sample preparation:** 0.5 mL Plasma or synovial fluid + 200 μL 2 M HCl + 5 mL hexane, tumble 10 min on a rotary mixer, centrifuge at 10 000 g for 5 min. Remove organic layer and evaporate it to dryness under vacuum centrifugation. Reconstitute residue in 150 μL MeOH + 100 μL water, vortex mix, inject aliquot.

### HPLC VARIABLES

**Guard column:** 20 × 2 Perisorb RP18 30-40 μm pellicular
**Column:** 125 × 4.6 5 μm Spherisorb ODS 1
**Mobile phase:** MeOH:water 63:37 adjusted to pH 3.3 with phosphoric acid
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 280

### CHROMATOGRAM

**Retention time:** 9
**Internal standard:** flurbiprofen
**Limit of detection:** <100 ng/mL

### OTHER SUBSTANCES

**Extracted:** diclofenac

**KEY WORDS**
plasma; flurbiprofen is IS

**REFERENCE**
Blagbrough,I.S.; Daykin,M.M.; Doherty,M.; Pattrick,M.; Shaw,P.N. High-performance liquid chromatographic determination of naproxen, ibuprofen and diclofenac in plasma and synovial fluid in man, *J.Chromatogr.*, **1992**, *578*, 251–257.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** 100 μL Serum or urine + 50 μL 1 M NaOH, let stand at room temperature for 20 min (to hydrolyse conjugates), add 50 μL 1 M HCl, add 1 mL 1 μg/mL IS in MeCN, add 2 mL 50 mM pH 2.6 potassium phosphate buffer, centrifuge at 2000 rpm for 10 min, inject 100 μL aliquot of supernatant.

**HPLC VARIABLES**
**Guard column:** 30 × 4.6 5 μm Brownlee RP-8
**Column:** 300 × 3.9 μBondapak C18
**Mobile phase:** THF:50 mM pH 2.6 potassium phosphate 45:55
**Flow rate:** 1.9
**Injection volume:** 100
**Detector:** F ex 360 em 320

**CHROMATOGRAM**
**Retention time:** 10
**Internal standard:** (RS)-2-(2-methoxy-4-biphenyl)propionic acid (8)
**Limit of quantitation:** 100 ng/mL

**KEY WORDS**
serum

**REFERENCE**
Adams,W.J.; Bothwell,B.E.; Bothwell,W.M.; VanGiessen,G.J.; Kaiser,D.G. Simultaneous determination of flurbiprofen and its major metabolite in physiological fluids using liquid chromatography with fluorescence detection, *Anal.Chem.*, **1987**, *59*, 1504–1509.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** 500 μL Urine + 100 μL 1 M NaOH (to hydrolyze conjugates), vortex. 500 μL Plasma or 600 μL basified urine + 200 μL 600 mM sulfuric acid, vortex, add 50 μL 100 μg/mL ketoprofen in water + 3 mL isooctane:isopropanol 96:5, mix vigorously for 45 s, centrifuge at 3000 rpm for 5 min. Remove the top layer and add it to 3 mL water, mix vigorously, centrifuge for 5 min, discard the organic layer. Add the aqueous layer to 350 μL 600 mM sulfuric acid, add 3 mL chloroform, mix, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL 50 mM triethylamine in MeCN, after 30 s add 50 μL 60 mM ethylchloroformate in MeCN, after 30 s add 50 μL 1 M L-leucinamide, after 2 min add 50 μL water, inject a 10-50 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 50 mm long 10 μm Partisil 5 ODS-3
**Column:** 100 × 4.6 5 μm Partisil 5 ODS-3
**Mobile phase:** MeCN:67 mM $KH_2PO_4$:triethylamine 35:65:0.02
**Flow rate:** 1
**Injection volume:** 10-50
**Detector:** UV 250

**CHROMATOGRAM**
**Retention time:** 17 (-), 21 (+)
**Internal standard:** ketoprofen (UV 275) (8 (-), 10 (+))
**Limit of quantitation:** 250 ng/mL (urine), 100 ng/mL (plasma)

**KEY WORDS**
plasma; derivatization; pharmacokinetics; chiral

**REFERENCE**
Berry,B.W.; Jamali,F. Stereospecific high-performance liquid chromatographic (HPLC) assay of flurbiprofen in biological specimens, *Pharm.Res.*, **1988**, *5*, 123–125.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 200.5

**CHROMATOGRAM**
**Retention time:** 21.337

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Serum. Activate an SPE cartridge filled with 100 mg 40-63 μm silica gel (Merck) with 1 mL MeOH and dry in a hot air oven at 100° for 1 h, equilibrate with 1 mL dichloromethane before use. 500 μL Serum + 100 μL 1 M HCl, mix, add 1 mL 1 M pH 3.8 sodium phosphate buffer, mix, add 3 mL diethyl ether, rock for 20 min, centrifuge at 1000 g for 2 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 100 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane, add 100 μL 1 mg/mL (R)-(+)-1-(1-naphthyl)ethylamine in dichloromethane, vortex briefly, let stand at room temperature for 2 h, add to the SPE cartridge, elute with two 1 mL portions of dichloromethane:MeCN 90:10. Combine all the eluate and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. Urine. Activate an SPE cartridge filled with 100 mg 40-63 μm silica gel (Merck) with 1 mL MeOH and dry in a hot air oven at 100° for 1 h, equilibrate with 1 mL dichloromethane before use. 500 μL Urine + 100 μL 1 M HCl, mix, add 1.5 mL 1 M pH 3.8 sodium phosphate buffer, mix, add 5 mL hexane:isopropanol 90:10, rock for 20 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 100 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane, add 100 μL 1 mg/mL (R)-(+)-1-(1-naphthyl)ethylamine in dichloromethane,

vortex briefly, let stand at room temperature for 2 h, add to the SPE cartridge, elute with two 1 mL portions of dichloromethane:MeCN 90:10. Combine all the eluate and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 10 × 2.1 40-63 μm pellicular C18 (Alltech)
**Column:** 150 × 3.9 5 μm Resolve C18 (Waters)
**Mobile phase:** MeCN:10 mM pH 3.5 phosphate buffer 50:50
**Flow rate:** 1.5
**Injection volume:** 50
**Detector:** F ex 290 em 330

**CHROMATOGRAM**
**Retention time:** 14.5 (R), 17.8 (S)
**Internal standard:** flurbiprofen

**OTHER SUBSTANCES**
**Interfering:** ibuprofen (a metabolite of ibuprofen interferes with the R enantiomer)

**KEY WORDS**
flurbiprofen is IS; derivatization; chiral; serum; SPE

**REFERENCE**
Tan,S.C.; Jackson,S.H.D.; Swift,C.G.; Hutt,A.J. Enantiospecific analysis of ibuprofen by high performance liquid chromatography: Determination of free and total drug enantiomer concentrations in serum and urine, *Chromatographia*, **1997**, *46*, 23–32.

**SAMPLE**
**Matrix:** bulk
**Sample preparation:** 10 mg Compound + 10 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride + 2 drops 3,5-dimethylaniline + 1.5 mL dichloromethane, mix, after 30 min add 1 mL 1 M HCl, shake vigorously. Remove the lower organic layer and dry it over anhydrous magnesium sulfate, inject an aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm D N-(3,5-dinitrobenzoyl)phenylglycine (Regis)
**Mobile phase:** Hexane:isopropanol 80:20
**Flow rate:** 2
**Injection volume:** 20
**Detector:** UV 254, UV 280

**CHROMATOGRAM**
**Retention time:** k' 2.27 (for first enantiomer)

**OTHER SUBSTANCES**
**Also analyzed:** carprofen, cicloprofen, etodolac, fenoprofen, ibuprofen, ketoprofen, naproxen, pirprofen, tiaprofenic acid

**KEY WORDS**
derivatization; $\alpha = 1.26$; chiral

**REFERENCE**
Pirkle,W.H.; Murray,P.G. The separation of the enantiomers of a variety of non-steroidal anti-inflammatory drugs (NSAIDS) as their anilide derivatives using a chiral stationary phase, *J.Liq.Chromatogr.*, **1990**, *13*, 2123–2134.

**SAMPLE**
**Matrix:** cell suspensions
**Sample preparation:** Centrifuge cell suspension at 2000 g for 4 min. Remove a 2 mL aliquot of the supernatant and add it to 200 μL 200 μg/mL IS in DMF, mix, add 200 μL 5 M HCl, extract

twice with 3 mL portions of toluene. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, add 20 μL 10 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 300 μL 10 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 300 μL 10 mg/mL (-)-(S)-α-methylbenzylamine in dichloromethane, let stand for 30 min, evaporate to dryness, reconstitute with 500 μL mobile phase, inject a 10 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 10 mm long Techsphere ODS (HPLC Technology, Macclesfield UK)
**Column:** 250 × 5 5 μm Techsphere ODS (HPLC Technology, Macclesfield UK)
**Mobile phase:** MeCN:7.5 mM $NaH_2PO_4$ 60:40, containing 5 mM sodium pentanesulfonate, pH adjusted to 2.8 with phosphoric acid
**Flow rate:** 1
**Injection volume:** 10
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** k' 4.75, 5.35 (enantiomers)
**Internal standard:** (S)-naproxen (k' 3.15)
**Limit of detection:** 500 ng/mL

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Thomason,M.J.; Hung,Y.-F.; Rhys-Williams,W.; Hanlon,G.W.; Lloyd,A.W. Indirect enantiomeric separation of 2-arylpropionic acids and structurally related compounds by reversed phase HPLC, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1765–1774.

**SAMPLE**
**Matrix:** dialysate
**Sample preparation:** Inject a 10 μL aliquot of dialysate (pH 7.4 isotonic phosphate buffer) containing 250 ng/mL naproxen.

**HPLC VARIABLES**
**Guard column:** 37-50 μm Corasil C18
**Column:** 100 × 4 5 μm Nucleosil C18
**Mobile phase:** MeCN:50 mM pH 3.0 phosphate buffer 48:52
**Flow rate:** 1.1
**Injection volume:** 10
**Detector:** F ex 258 em 310

**CHROMATOGRAM**
**Retention time:** 4
**Internal standard:** naproxen (F ex 262 em 356) (2.5)

**KEY WORDS**
mouse; rat; pharmacokinetics

**REFERENCE**
Evrard,P.A.; Deridder,G.; Verbeeck,R.K. Intravenous microdialysis in the mouse and the rat: Development and pharmacokinetic application of a new probe, *Pharm.Res.*, **1996**, *13*, 12–17.

**SAMPLE**
**Matrix:** microsomal incubations
**Sample preparation:** 300 μL Microsomal incubation + 20 μL 6 M HCl, centrifuge at 3000 g for 10 min, inject a 100 μL aliquot of the supernatant.

**HPLC VARIABLES**
**Guard column:** μBondapak C18
**Column:** 250 × 4 5 μm Lichrospher RP-18

**Mobile phase:** MeCN:water:trifluoroacetic acid 65:145:0.08
**Column temperature:** 37
**Flow rate:** 1.5
**Injection volume:** 100
**Detector:** UV 273

**CHROMATOGRAM**
**Retention time:** 70
**Internal standard:** 1-naphthol-β-D-glucuronide

**OTHER SUBSTANCES**
**Extracted:** metabolites glucuronides

**KEY WORDS**
rat; human; liver

**REFERENCE**
Hamdoune,M.; Mounie,J.; Magdalou,J.; Masmoudi,T.; Goudonnet,H.; Escousse,A. Characterization of the in vitro glucuronidation of flurbiprofen enantiomers, *Drug Metab.Dispos.*, **1995**, *23*, 343–348.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 Spherisorb S10-ODS2
**Mobile phase:** MeCN:water:acetic acid 60:35:0.5
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 280

**OTHER SUBSTANCES**
**Simultaneous:** mefenamic acid

**REFERENCE**
Galia,E.; Nicolaides,E.; Hörter,D.; Löbenberg,R.; Reppas,C.; Dressman,J.B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, *Pharm.Res.*, **1998**, *15*, 698–705.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Dilute in an appropriate solvent, inject an aliquot.

**HPLC VARIABLES**
**Guard column:** RC18 Guardpak (Waters)
**Column:** 150 × 4.5 5 μm Altex C18
**Mobile phase:** MeOH:water:acetic acid 67:32.5:0.5
**Flow rate:** 1.5
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 8

**REFERENCE**
Richman,J.B.; Tang-Liu,D.D.-S. A corneal perfusion device for estimating ocular bioavailability in vitro, *J.Pharm.Sci.*, **1990**, *79*, 153–157.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Mix 100 μL of a 1-200 μM solution of carboxylic acid in dichloromethane with 100 μL 800 μM (-)-APMB in dichloromethane, 100 μL 1.6 mM 2,2'-dipyridyl disulfide in dichloromethane, and 100 μL 1.6 mM triphenylphosphine in dichloromethane, let stand at

room temperature for 20 min. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 400 μL mobile phase, inject a 10 μL aliquot. ((-)-APMB is (-)-2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole. Synthesis is as follows. Hydrogenate 5-methoxy-2-nitrophenol in EtOH over platinum oxide to give 2-amino-5-methoxyphenol (J. Org. Chem. 1957, 22, 220). It should be possible to prepare ethyl 4-acetylbenzimidate hydrochloride ($CH_3COC_6H_4C(=NH)OC_2H_5.HCl$) by passing dry hydrogen chloride into a mixture of 4-acetylbenzonitrile and 1.2-1.5 equivalents EtOH in an inert solvent (e.g., benzene, chloroform, dioxane, ether, nitrobenzene (Caution! Benzene, chloroform, and dioxane are carcinogens!) at 0-5°, the benzimidate should crystallize from the mixture in 7-10 days (J. Chem. Soc. 1942, 103). Add a solution of 5.5 g 2-amino-5-methoxyphenol in 200 mL MeOH to 9 g ethyl 4-acetylbenzimidate hydrochloride, stir at 60-70° for 4 h, evaporate to dryness under reduced pressure, recrystallize from EtOH to give 4-(6-methoxy-2-benzoxazolyl)acetophenone as fine orange-yellow crystals (mp 167°) (J. Chromatogr. 1990, 532, 65). Add 7.0 g hydroxylamine hydrochloride and 8.2 g sodium acetate to 10.1 g 4-(6-methoxy-2-benzoxazolyl)acetophenone in 500 mL EtOH:water 95:5, reflux for 1 h, pour into ice-water, filter, recrystallize from EtOH:water 90:10 to give 4-(6-methoxy-2-benzoxazolyl)acetophenone oxime as faint reddish needles (mp 212°). Dissolve 4.7 g 4-(6-methoxy-2-benzoxazolyl)acetophenone oxime in 300 mL MeOH, add 3 g 10% palladium on charcoal, add 10.5 g ammonium formate, reflux for 30 min, filter, evaporate the filtrate to dryness under reduced pressure. Take up the residue in 100 mL 5% HCl and wash the aqueous phase with 100 mL ethyl acetate. Adjust the pH of the aqueous layer to 13-14 with 10% NaOH and extract with 200 mL ethyl acetate. Wash the organic layer with 100 mL water and dry it over anhydrous sodium sulfate, evaporate to dryness under reduced pressure to give racemic 2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole. Dissolve 3.6 g racemic 2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole in 50 mL EtOH and add 3.5 g (S)-(-)-α-methoxy-α-trifluoromethylphenylacetic acid, allow to stand overnight at 5°. Collect the precipitate and fractionally crystallize it from EtOH 4 times. Take up the final product in 5% NaOH and extract it with ethyl acetate, wash the organic layer with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from EtOH to give (-)-2-[4-(1-aminoethyl)phenyl]-6-methoxybenzoxazole as pale yellow crystals (mp 74°).)

## HPLC VARIABLES

**Column:** 150 × 4.6 5 μm TSK gel ODS-80TM (Tosoh)
**Mobile phase:** MeCN:water:acetic acid 60:40:0.1
**Flow rate:** 1
**Injection volume:** 10
**Detector:** F ex 320 em 380

## CHROMATOGRAM

**Retention time:** 16 (S), 18 (R)
**Limit of detection:** 10 fmole

## OTHER SUBSTANCES

**Simultaneous:** ibuprofen, naproxen

## KEY WORDS

derivatization; chiral

## REFERENCE

Kondo,J.; Imaoka,T.; Kawasaki,T.; Nakanishi,A.; Kawahara,Y. Fluorescence derivatization reagent for resolution of carboxylic enantiomers by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *645*, 75–81.

## SAMPLE

**Matrix:** solutions
**Sample preparation:** Mix 100 μL of a solution of the carboxylic acid in MeCN with 100 μL 2 mM (S)-1-(4-dansylaminophenyl)ethylamine in MeCN, add 100 μL 3 mM 2,2'-dipyridyl disulfide (Aldrithiol-2) in MeCN, add 100 μL 3 mM triphenylphosphine in MeCN, vortex, let stand at room temperature for 3 h, inject a 5 μL aliquot. (Synthesis of (S)-1-(4-dansylaminophenyl)ethylamine is as follows. Add 2.2 g di-tert-butyl dicarbonate dropwise to a stirred solution of 2 g (S)-α-methyl-4-nitrobenzylamine hydrochloride ((S)-1-(4-nitrophenyl)ethylamine hydrochloride) and 1.1 g triethylamine in 20 mL MeCN at 0°, stir at room temperature for 1 h, evaporate to dryness under reduced pressure. Dissolve the residue in 50 mL ethyl acetate and wash with 10% aqueous citric acid, wash with water, dry over anhydrous sodium sulfate,

evaporate to dryness under reduced pressure to give (S)-N-tert-butoxycarbonyl-1-(4-nitrophenyl)ethylamine as white crystals (mp 86-89°). Add 200 mg 5% PdC to a solution of 2 g (S)-N-tert-butoxycarbonyl-1-(4-nitrophenyl)ethylamine in 40 mL MeOH, stir, hydrogenate at room temperature for 3 h, filter, evaporate the filtrate to dryness to obtain (S)-N-tert-butoxycarbonyl-1-(4-aminophenyl)ethylamine. Stir 1.4 g (S)-N-tert-butoxycarbonyl-1-(4-aminophenyl) ethylamine in 10 mL MeCN and 50 mL 100 mM pH 9.0 sodium bicarbonate, add a solution of 1.9 g dansyl chloride in 30 mL MeCN dropwise while maintaining the pH of the solution at 9.0 with 1 M NaOH, stir at room temperature for 20 min, stir at 45° for 2 h, cool to room temperature, extract three times with 30 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under reduced pressure, recrystallize the residue from benzene/hexane to obtain (S)-N-tert-butoxycarbonyl-1-(4-dansylaminophenyl)ethylamine as pale-yellow crystals (mp 97-101°) (Caution! Benzene is a carcinogen!). Add 2 mL concentrated HCl to a solution of 1.5 g (S)-N-tert-butoxycarbonyl-1-(4-dansylaminophenyl)ethylamine in 10 mL MeOH, stir at room temperature for 30 min, evaporate to dryness under reduced pressure. Dissolve the residue in 30 mL water and adjust the pH to 8.0 with sodium bicarbonate, extract three times with 30 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under reduced pressure, recrystallize the residue from EtOH to obtain (S)-1-(4-dansylaminophenyl)ethylamine as pale-yellow crystals (mp 157-160°; $[\alpha]_D^{28}$ -11.1° (c = 0.2 in MeCN)). (The (R)-enantiomer can be prepared in an exactly analogous fashion.))

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**HPLC VARIABLES**
**Column:** 150 × 4.6 5 μm ODS-80TM (Tosoh)
**Mobile phase:** MeCN:50 mM pH 6.5 sodium acetate buffer 65:35
**Flow rate:** 1
**Injection volume:** 5
**Detector:** F ex 338 em 535

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**CHROMATOGRAM**
**Retention time:** k' 8.71, k' 10.57 (enantiomers)
**Limit of detection:** 170 fmole

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**OTHER SUBSTANCES**
**Also analyzed:** ibuprofen, phenoprofen, 2-phenylpropionic acid, pranoprofen, naproxen

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**KEY WORDS**
derivatization; chiral

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**REFERENCE**
Iwaki,K.; Bunrin,T.; Kameda,Y.; Yamazaki,M. Resolution and sensitive detection of carboxylic acid enantiomers using fluorescent chiral derivatization reagents by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *662*, 87–93.

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**SAMPLE**
**Matrix:** solutions

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**HPLC VARIABLES**
**Column:** 250 × 4.6 10 μm Chiralpak AD (Daicel)
**Mobile phase:** Carbon dioxide:MeOH 96:4
**Column temperature:** 30
**Flow rate:** 2.5
**Detector:** UV 210

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**CHROMATOGRAM**
**Retention time:** 10, 17 (enantiomers)

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**OTHER SUBSTANCES**
**Simultaneous:** fenoprofen, ibuprofen, ketoprofen, naproxen

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**KEY WORDS**
SFC; 250 bar; chiral

**REFERENCE**
Kot,A.; Sandra,P.; Venema,A. Sub- and supercritical fluid chromatography on packed columns: A versatile tool for the enantioselective separation of basic and acidic drugs, *J.Chromatogr.Sci.*, **1994**, *32*, 439–448.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** Ultrasphere ODS
**Mobile phase:** MeOH:water:acetic acid 67:32.5:0.5
**Flow rate:** 1.5
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 7
**Limit of detection:** 2 ng

**OTHER SUBSTANCES**
**Simultaneous:** flurbiprofen amide

**KEY WORDS**
rabbit; buffer

**REFERENCE**
Tang-Liu,D.D.-S.; Richman,J.B.; Weinkam,R.J.; Takruri,H. Effects of four penetration enhancers on corneal permeability of drugs in vitro, *J.Pharm.Sci.*, **1994**, *83*, 85–90.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Mix 1 mL 100 μg/mL compound in dichloromethane with 300 μL 100 μg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, 300 μL 1.1 mg/mL 1-ethyl-3-dimethylaminopropylcarbodiimide hydrochloride in dichloromethane, and 300 μL 300 μg/mL benzylamine in dichloromethane, vortex, let stand at room temperature for 1.5 h, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL MeOH, inject an aliquot.

**HPLC VARIABLES**
**Column:** 150 × 4.6 10 μm EXP B101 tris(4-methylbenzoate) cellulose on silica (Bio-Rad)
**Mobile phase:** MeOH:buffer 70:30 (Prepare buffer solution by dissolving 14.05 g sodium perchlorate in water, adjust pH to 2.0, make up to 1 L with water.)
**Flow rate:** 1
**Detector:** UV 230

**CHROMATOGRAM**
**Retention time:** 27, 35 (enantiomers)

**OTHER SUBSTANCES**
**Also analyzed:** benoxaprofen (MeOH:buffer 80:20), carprofen, fenoprofen, ibuprofen, ketoprofen, pirprofen, tiaprofenic acid

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Van Overbeke,A.; Baeyens,W.; Van den Bossche,W.; Dewaele,C. Separation of 2-arylpropionic acids on a cellulose based chiral stationary phase by RP-HPLC, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 901–909.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Mix 1 mL 100 μg/mL compound in dichloromethane with 300 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, 300 μL 1 mg/mL 1-ethyl-3-di-

methylaminopropylcarbodiimide hydrochloride in dichloromethane, and 300 μL 1-1.5 mg/mL benzylamine in dichloromethane, vortex, let stand at room temperature for 1.5 h, wash with 1 mL 250 mM HCl, wash with 1 mL water. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

**HPLC VARIABLES**
**Column:** 10 μm EXP B101 4-methylbenzoate cellulose on silica gel(Bio-Rad)
**Mobile phase:** MeOH:50 mM pH 1.5 perchlorate buffer 80:20
**Flow rate:** 1
**Detector:** UV 230

**CHROMATOGRAM**
**Retention time:** 7, 8

**OTHER SUBSTANCES**
**Simultaneous:** ketoprofen

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Van Overbeke,A.; Baeyens,W.; Van den Bossche,W.; Dewaele,C. Enantiomeric separation of amide derivatives of some 2-arylpropionic acids by HPLC on a cellulose-based chiral stationary phase, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 911–916.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 125 × 3 Ecocart LiChrospher 100 RP-18
**Mobile phase:** Isopropanol:100 mM $KH_2PO_4$:formic acid 54:100:0.1
**Flow rate:** 0.6
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 12.0
**Limit of quantitation:** 200-500 ng/mL

**OTHER SUBSTANCES**
**Simultaneous:** acemetacin; diclofenac; indomethacin; lonazolac; ketoprofen; naproxen; piroxicam; sulindac; tenoxicam

**REFERENCE**
Baeyens,W.R.G.; Van Der Weken,G.; Van Overbeke,A.; Zhang,Z.D. Preliminary results on the LC-separation of non-steroidal anti-inflammatory agents in conventional and narrow-bore RP set-ups applying columns with different internal diameters, *Biomed.Chromatogr.*, **1995**, *9*, 261–262.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
**Flow rate:** 0.6
**Injection volume:** 25
**Detector:** UV 229

**CHROMATOGRAM**
**Retention time:** 8.01 (A), 8.91 (B)

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

## KEY WORDS

details of plasma extraction

## REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

## SAMPLE

**Matrix:** solutions
**Sample preparation:** Prepare a 100 μM solution in buffer, inject a 20 μL aliquot.

## HPLC VARIABLES

**Column:** 100 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)
**Mobile phase:** 50 mM pH 5.5 $KH_2PO_4$
**Flow rate:** 0.8
**Injection volume:** 20
**Detector:** UV

## CHROMATOGRAM

**Retention time:** k' 6.10

**OTHER SUBSTANCES**
**Simultaneous:** isradipine, ketoprofen, nimodipine, suprofen

**KEY WORDS**
chiral; $\alpha$ = 1.13

**REFERENCE**
Massolini,G.; De Lorenzi,E.; Ponci,M.C.; Gandini,C.; Caccialanza,G.; Monaco,H.L. Egg yolk riboflavin binding protein as a new chiral stationary phase in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 55–65.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** 1 mL 5 mM flurbiprofen in dichloromethane + 300 µL 1 mg/mL hydroxybenzotriazole in dichloromethane:pyridine 99:1 + 300 µL 11 mg/mL 1-ethyl-3-dimethylaminopropylcarbodiimide in dichloromethane + 300 µL 3.47 mg/mL 1-naphthylamine (Caution! 1-Naphthylamine in a carcinogen!) in dichloromethane, vortex, let stand for 1 h, evaporate to dryness under a stream of nitrogen, reconstitute with 5 mL MeOH, inject an aliquot.

**HPLC VARIABLES**
**Column:** 150 × 2.1 Tolycellulose EXP B101 (tris(4-methylbenzoate)cellulose covalently bonded to 10 µm aminopropylsilica)
**Mobile phase:** MeOH:buffer 85:15 (Buffer was 14.05 g/L sodium perchlorate adjusted to pH 2.0.)
**Flow rate:** 0.21
**Injection volume:** 1
**Detector:** UV 230, UV 254

**CHROMATOGRAM**
**Retention time:** k' 6.46 (first enantiomer)

**OTHER SUBSTANCES**
**Also analyzed:** fenoprofen, ibuprofen, ketoprofen, tiaprofenic acid

**KEY WORDS**
derivatization; narrow-bore; chiral; $\alpha$=2.18; (see Biomed. Chromatogr. 1995; 9; 292)

**REFERENCE**
Van Overbeke,A.; Baeyens,W.; Van Der Weken,G.; Van de Voorde,I.; Dewaele,C. Comparative chromatographic study on the chiral separation of the 1-naphthylamine derivative of ketoprofen on cellulose-based columns of different sizes, *Biomed.Chromatogr.*, **1995**, *9*, 289–290.

**SAMPLE**
**Matrix:** urine
**Sample preparation:** Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 µL 20 µg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 µL 1 M pH 5.0 acetate buffer, vortex. Add 250 µL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 µL mobile phase, inject a 10-30 µL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 4.6 5 µm Inertsil ODS-2
**Mobile phase:** MeCN:50 mM pH 5.0 phosphate buffer 42:58
**Flow rate:** 0.9
**Injection volume:** 10-30
**Detector:** UV 230

**CHROMATOGRAM**
**Retention time:** 15
**Internal standard:** indomethacin (18.5)
**Limit of quantitation:** 50 ng/mL

**OTHER SUBSTANCES**
**Extracted:** diclofenac, ibuprofen, felbinac, fenbufen, ketoprofen, loxoprofen, mefenamic acid, naproxen, piroxicam, sulindac

**KEY WORDS**
SPE

**REFERENCE**
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

**SAMPLE**
**Matrix:** urine
**Sample preparation:** 1 mL Urine + 1 mL 6 M HCl, heat at 90° for 30 min, cool to room temperature, add ibuprofen (15 μg per 100 μL), add 5 mL dichloroethane, mix 15 min, centrifuge at 800 g for 5-10 min. Evaporate organic layer under a stream of nitrogen at 37-40°. Reconstitute with 300 μL chloroform, add 200 μL 65 mg/mL 1,1'-carbonyldiimidazole in chloroform, let stand 5-10 min at room temperature, add 10 μL glacial acetic acid, vortex briefly, let stand 5-10 min at room temperature, add 50 μL S-(α)-methylbenzylamine, mix briefly, let stand for 30 min at room temperature, add 3 mL 0.25 M ammonium hydroxide, add 5 mL dichloroethane, mix gently for 15 min. Remove organic layer and wash it with 3 mL 0.25 M ammonium hydroxide and twice with 3 mL 1 M HCl (with 15 min mixing each time). Evaporate organic layer under a stream of nitrogen at 37°, dissolve in 150 μL MeCN:water 45:55, inject 20-30 μL aliquot.

**HPLC VARIABLES**
**Guard column:** Brownlee RP18
**Column:** 250 × 4.6 5 μm Ultrasphere ODS
**Mobile phase:** MeCN:50 mM acetic acid 55:45
**Flow rate:** 1
**Injection volume:** 20-30
**Detector:** F ex 200 em 320 (cut-off) (flurbiprofen), UV 232 (ibuprofen)

**CHROMATOGRAM**
**Retention time:** 17 (S), 19 (R)
**Internal standard:** ibuprofen (24 S, 27 R)
**Limit of detection:** 10 ng/mL
**Limit of quantitation:** 25 ng/mL

**OTHER SUBSTANCES**
**Simultaneous:** metabolites

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Knadler,M.P.; Hall,S.D. High-performance liquid chromatographic analysis of the enantiomers of flurbiprofen and its metabolites in plasma and urine, *J.Chromatogr.*, **1989**, *494*, 173–182.

**SAMPLE**
**Matrix:** urine
**Sample preparation:** Dilute urine 20-fold with 100 mM pH 2.0 phosphate buffer, extract twice with two volumes of ethyl acetate, centrifuge at 5000 g for 5 min. Combine the organic layers and evaporate them to dryness under a stream of nitrogen below 30°. Reconstitute in 0.2-1 mL mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Guard column:** 4 × 4 5 μm LiChrospher 100 RP-18
**Column:** 250 × 4 5 μm LiChrospher CH-18

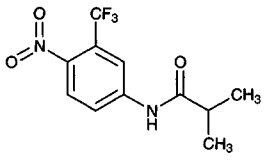
**Mobile phase:** MeOH:10 mM pH 6.0 phosphate buffer 80:20 containing 2.5 mM cethexonium bromide (Rinse with 100 mL MeOH:EtOH:water 50:25:25 at the end of the day.)
**Flow rate:** 1
**Injection volume:** 10
**Detector:** UV 273

### CHROMATOGRAM
**Retention time:** k' 4.1

### OTHER SUBSTANCES
**Extracted:** glucuronides, pirprofen

### REFERENCE
Liu,H.-F.; Leroy,P.; Nicolas,A.; Magdalou,J.; Siest,G. Evaluation of a versatile reversed-phase high-performance liquid chromatographic system using cethexonium bromide as ion-pairing reagent for the analysis of glucuronic acid conjugates, *J.Chromatogr.*, **1989**, *493*, 137–147.

# Flutamide



**Molecular formula:** $C_{11}H_{11}F_3N_2O_3$
**Molecular weight:** 276.22
**CAS Registry No.:** 13311-84-7
**Merck Index:** 4242
**Lednicer No.:** 3 57

### SAMPLE
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

### HPLC VARIABLES
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 200.5

### CHROMATOGRAM
**Retention time:** 22.248

### KEY WORDS
whole blood

### REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** microsomal incubations
**Sample preparation:** Vigorously mix 500 μL microsomal incubation with 5 mL dichloromethane. Evaporate the dichloromethane layer under a stream of nitrogen at 30°. Reconstitute the residue in 100 μL MeOH. Inject an aliquot.

**HPLC VARIABLES**
**Column:** 300 × 3.9 CP-18 μBondapak
**Mobile phase:** Gradient. A was MeOH:water 60:40. B was MeOH. A:B from 100:0 to 60:40 over 40 min, flush column with MeOH for 10 min, re-equilibrate at initial conditions
**Flow rate:** 1
**Detector:** UV; Radioactivity (Radiometer Flo-1)

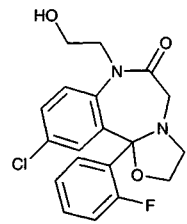
**CHROMATOGRAM**
**Retention time:** 18.3

**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
radiolabeled

**REFERENCE**
Shet,M.D.; McPhaul,M.; Fisher,C.W.; Stallings,N.R.; Estabrook,R.W. Metabolism of the antiandrogenic drug (flutamide) by human CYP1A2, *Drug Metab.Dispos.*, **1997**, *25*, 1298–1303.

# Flutazolam




**Molecular formula:** $C_{19}H_{18}ClFN_2O_3$
**Molecular weight:** 376.81
**CAS Registry No.:** 27060-91-9
**Merck Index:** 4243

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase in a water bath under a stream of nitrogen at 40°. Dissolve residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

**HPLC VARIABLES**
**Column:** 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
**Mobile phase:** MeCN:5 mM pH 6 $NaH_2PO_4$ 45:55
**Flow rate:** 0.65
**Injection volume:** 20
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 32.2 (A), 97.4 (B)
**Internal standard:** diazepam (29.8 (A), 77.5 (B))
**Limit of quantitation:** 10 ng/mL (A)

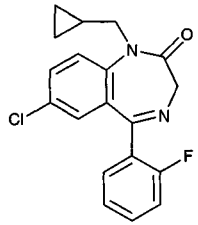
**OTHER SUBSTANCES**
**Extracted:** bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam
**Simultaneous:** alprazolam

**Noninterfering:** barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

**KEY WORDS**
serum

**REFERENCE**
Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

# Flutoprazepam




**Molecular formula:** $C_{15}H_{16}ClFN_2O$
**Molecular weight:** 342.80
**CAS Registry No.:** 25967-29-7
**Merck Index:** 4245

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 200 μL Plasma + 25 μL 1 μg/mL o-chlorodiazepam in MeOH, vortex gently, extract twice with 1 mL portions of benzene (Caution! Benzene is a carcinogen!) with shaking. Combine the extracts evaporate them to dryness under a stream of nitrogen at 30-35°, reconstitute the residue in 100 μL mobile phase, inject a 10-90 μL aliquot.

**HPLC VARIABLES**
**Column:** 300 × 3.9 10 μm μBondapak C18
**Mobile phase:** MeCN:10 mM $KH_2PO_4$ 50:50 adjusted to pH 4.5 with orthophosphoric acid
**Flow rate:** 1.2
**Injection volume:** 10-90
**Detector:** UV 229

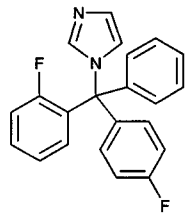
**CHROMATOGRAM**
**Retention time:** 16
**Internal standard:** o-chlorodiazepam
**Limit of detection:** 25 ng/mL

**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
plasma; rat; pharmacokinetics

**REFERENCE**
Conti,I.; Sarati,S.; Caccia,S. Propranolol does not alter flutoprazepam kinetics and metabolism in the rat, *Eur.J.Drug Metab.Pharmacokinet.*, **1991**, *16*, 53–58.

# Flutrimazole



**Molecular formula:** $C_{22}H_{16}F_2N_2$
**Molecular weight:** 346.38
**CAS Registry No.:** 119006-77-8
**Merck Index:** 4247

**SAMPLE**
**Matrix:** formulations, tissue
**Sample preparation:** Formulations. 500 mg Cream + 30 mL EtOH, heat mixture to 50° to melt it, cool, dilute to 50 mL, filter through a 0.45 μm filter. Inject a 25 μL aliquot of the filtrate. Tissue. Add 3 mL MeOH to cleaned and cut skin, sonicate for 15 min, inject an aliquot.

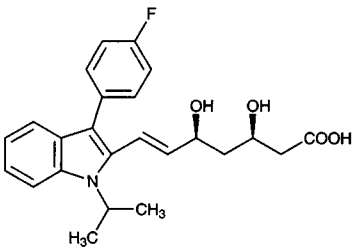
**HPLC VARIABLES**
**Guard column:** 10 μm μBondapak C18
**Column:** 10 μm μBondapak C18
**Mobile phase:** MeOH:pH 7.0 phosphate buffer 80:20
**Flow rate:** 1
**Injection volume:** 25
**Detector:** UV 225

**CHROMATOGRAM**
**Limit of quantitation:** 50 ng/mL

**KEY WORDS**
cream; skin

**REFERENCE**
Ramis,J.; Conte,L.; Segado,X.; Forn,J.; Lauroba,J.; Calpena,A.; Escribano,E.; Domenech,J. Influence of formulation on the in vitro transdermal penetration of flutrimazole, *Arzneimittelforschung*, **1997**, *47*, 1139–1144.

# Fluvastatin



**Molecular formula:** $C_{24}H_{26}FNO_4$
**Molecular weight:** 411.47
**CAS Registry No.:** 93957-54-1, 93957-55-2 (sodium salt)
**Merck Index:** 4250
**Lednicer No.:** 5 105

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Mix 500 μL plasma with 500 μL MeCN and 500 μL pH 6.0 phosphate buffer, extract with 5 mL MTBE by shaking for 30 min, centrifuge at 1200 g for 5 min, evaporate the organic phase under nitrogen. Dissolve the residue in 400 μL MeCN:water 40:60, vortex 3 times for 30 s, inject a 20-300 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 15 × 3.2 7 μm Brownlee Si
**Column:** 250 × 4.6 10 μm Chiralcel OD-R (Daicel Chemical Industries, Japan)
**Mobile phase:** MeCN:buffer 40:60 (Buffer was pH 2.5 phosphate buffer (I = 0.04).)
**Column temperature:** 15
**Flow rate:** 0.5

**Injection volume:** 100
**Detector:** F ex 305 em 390

**CHROMATOGRAM**
**Retention time:** 28.0 (3S, 5R), 30.0 (3R, 5S)
**Limit of quantitation:** 5 nM

**KEY WORDS**
chiral; plasma

**REFERENCE**
Toreson,H.; Eriksson,B.-M. Determination of fluvastatin enantiomers and the racemate in human blood plasma by liquid chromatography and fluorimetric detection, *J.Chromatogr.A*, **1996**, *729*, 13–18.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Mix 500 μL plasma with 100 μL 500 nM IS, 500 μL MeCN, and 500 μL pH 6.0 phosphate buffer, extract with 5 mL MTBE by shaking for 30 min, centrifuge at 1200 g for 5 min, evaporate the organic phase under nitrogen. Dissolve the residue in 400 μL mobile phase, vortex 3 times for 30 s, inject an aliquot.

**HPLC VARIABLES**
**Guard column:** 15 × 3.2 7 μm Brownlee CN
**Column:** 150 × 4.6 5 μm Zorbax Rx-C8
**Mobile phase:** MeOH:pH 6.0 phosphate buffer:100 mM tetrabutylammonium fluoride 60:25:15
**Column temperature:** 40
**Flow rate:** 1
**Injection volume:** 50
**Detector:** F ex 305 em 390 following post-column photolysis. The column effluent flowed through a knitted 10 m × 0.3 mm PTFE coil irradiated at 254 nm to the detector.

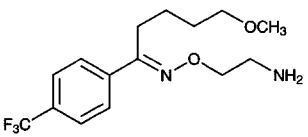
**CHROMATOGRAM**
**Retention time:** 15.0
**Internal standard:** Sandoz compound 63-267 ([R,S,-(E)-] (±)-7-(3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2yl)-(3,5-dihydroxy-6-methyl)-6-heptenoic acid, monosodium salt) (20.5)
**Limit of quantitation:** 500 pM

**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
plasma; post-column reaction; post-column photochemical derivatization

**REFERENCE**
Toreson,H.; Eriksson,B.-M. Determination of fluvastatin enantiomers and the racemate in human blood plasma by liquid chromatography and fluorimetric detection, *J.Chromatogr.A*, **1996**, *729*, 13–18.

# Fluvoxamine




**Molecular formula:** $C_{15}H_{21}F_3N_2O_2$
**Molecular weight:** 318.34
**CAS Registry No.:** 54739-18-3
**Merck Index:** 4251

**SAMPLE**
**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 100 μL 900 ng/mL IS + 4 mL 300 mM trisodium phosphate, extract with 400 μL diisopropyl ether for 20 min, centrifuge. Evaporate upper organic layer to dryness under a stream of nitrogen, dissolve residue in 100 μL MeCN , inject a 50 μL aliquot. (Caution! Diisopropyl ether readily forms explosive peroxides!)

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**HPLC VARIABLES**
**Column:** 150 × 4.6 3 μm Supelcosil LC-SI
**Mobile phase:** MeCN:MeOH:concentrated ammonia 87.5:12.0:0.5
**Flow rate:** 2
**Injection volume:** 50
**Detector:** UV 254

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**CHROMATOGRAM**
**Retention time:** 2.1
**Internal standard:** 5-(pyrrolidinylpropyliden)-10,11-dihydro-5H-dibenzo[a,d]cyclohepten (4.1)
**Limit of detection:** 2.2 nM

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**OTHER SUBSTANCES**
**Noninterfering:** amitriptyline, citalopram, clomipramine, desmethylclomipramine, imipramine, nortriptyline, risperidone

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**KEY WORDS**
plasma; pharmacokinetics

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**REFERENCE**
Carrillo,J.A.; Dahl,M.-L.; Svensson,J.-O.; Alm,C.; Rodríguez,I.; Bertilsson,L. Disposition of fluvoxamine in humans is determined by the polymorphic CYP2D6 and also by the CYP1A2 activity, *Clin.Pharmacol.Ther.*, **1996**, *60*, 183–190.

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**SAMPLE**
**Matrix:** blood
**Sample preparation:** 1 mL Plasma + 20 μL 10 μg/mL clovoxamine + 120 μL 2 M NaOH + 4 mL heptane:isopropanol 98:2, shake for 30 min, centrifuge at 3000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, shake for 20 min, centrifuge at 3000 g for 10 min, inject a 20 μL aliquot of the aqueous layer.

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**HPLC VARIABLES**
**Column:** 120 × 4.6 5 μm Nucleosil C8
**Mobile phase:** MeCN:buffer 36:64 (Buffer was 16 mM $KH_2PO_4$ adjusted to pH 2.5 with concentrated phosphoric acid.)
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 215

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**CHROMATOGRAM**
**Retention time:** 4.3
**Internal standard:** clovoxamine (3.3)
**Limit of detection:** 25 ng/mL

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**OTHER SUBSTANCES**
**Simultaneous:** amitriptyline, chlorimipramine, desipramine, doxepin, imipramine, nortriptyline, trimipramine

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**KEY WORDS**
plasma

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**REFERENCE**
Foglia,J.P.; Birder,L.A.; Perel,J.M. Determination of fluvoxamine in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1989**, *495*, 295–302.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 200 μL 1 μg/mL metapramine in MeOH + 2 mL 1 M pH 10.0 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 4000 g for 5 min. Remove the organic layer and add it to 2 mL 62.5 mM sulfuric acid, vortex for 5 min, centrifuge at 4000 g for 5 min. Remove the aqueous phase and add it to 1 mL 500 mM NaOH, vortex, add 6 mL hexane:diethyl ether 50:50, shake for 10 min, centrifuge at 4000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 mM sodium carbonate, add 10 μL 10 mg/mL dansyl chloride in acetone, vortex for 1 min, heat at 45° for 30 min, evaporate under a stream of nitrogen at 50°. Reconstitute the residue in 200 μL MeCN:water 45:55, inject a 100 μL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.6 5 μm Hypersil ODS

**Mobile phase:** Gradient. MeCN:water from 45:55 to 65:35 over 10 min, maintain at 65:35 for 20 min

**Column temperature:** 30

**Flow rate:** 1.5

**Injection volume:** 100

**Detector:** F (Fluorichrom ex 7.54 and 7.60 filters, em 3.71 and 4.76 filters

## CHROMATOGRAM

**Retention time:** 19

**Internal standard:** metapramine (28)

**Limit of detection:** 1.5 ng/mL

## OTHER SUBSTANCES

**Noninterfering:** alimemazine, alprazolam, amineptine, amitriptyline, caffeine, clobazam, clomipramine, clorazepate, cyamemazine, diazepam, demethyldiazepam, flunitrazepam, levomepromazine, loprazolam, lorazepam, meprobamate, nitrazepam, oxazepam, triazolam, viloxazine

## KEY WORDS

plasma; protect from light; derivatization

## REFERENCE

Pommery,J.; Lhermitte,M. High performance liquid chromatographic determination of fluvoxamine in human plasma, *Biomed.Chromatogr.*, **1989**, *3*, 177–179.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 500 μL 2 M sodium bicarbonate + 100 μL 0.1 or 1 μg/mL clovoxamine in MeOH + 10 mL hexane, shake horizontally for 20 min, centrifuge at 2000 g for 10 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

## HPLC VARIABLES

**Column:** 150 × 3.9 5 μm Resolve spherical silica (Waters)

**Mobile phase:** MeOH:MeCN:THF:water:diethylamine 98.59:1:0.2:0.2:0.01

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 4.7

**Internal standard:** clovoxamine (5.2)

**Limit of detection:** 0.5 ng/mL

**Limit of quantitation:** 2 ng/mL

## OTHER SUBSTANCES

**Simultaneous:** alimemazine, amitriptyline, chlorpromazine, clomipramine, desipramine, fluoxetine, haloperidol, imipramine, levomepromazine, norfluoxetine, nortriptyline, propericiazine, trimipramine, viloxazine

**Noninterfering:** metabolites, amineptine, buspirone, clobazam, clonazepam, clorazepate, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam
**Interfering:** imipramine

### KEY WORDS
plasma; human; rat; normal phase; pharmacokinetics

### REFERENCE
Van Der Meersch-Mougeot,V.; Diquet,B. Sensitive one-step extraction procedure for column liquid chromatographic determination of fluvoxamine in human and rat plasma, *J.Chromatogr.*, **1991**, *567*, 441–449.

### SAMPLE
**Matrix:** blood
**Sample preparation:** Add 10 μL 20 μg/mL oxaprotiline in MeOH to 990 μL plasma or serum. Inject 100 μL plasma or serum onto column A with mobile phase A and elute to waste, after 15 min elute column A onto column B with mobile phase B for 2 min. Remove column A from circuit and re-equilibrate it with mobile phase A for 5 min. Chromatograph on column B with mobile phase B.

### HPLC VARIABLES
**Column:** A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN
**Mobile phase:** A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:578:235
**Flow rate:** 1.5
**Injection volume:** 100
**Detector:** UV 214

### CHROMATOGRAM
**Retention time:** 6.2
**Internal standard:** oxaprotiline (9.5)
**Limit of detection:** 20 ng/mL

### OTHER SUBSTANCES
**Simultaneous:** metoclopramide, doxepin, amitriptyline, clomipramine, fluoxetine, imipramine, norfluoxetine, nortriptyline, desipramine, maprotiline
**Noninterfering:** haloperidol, spiroperidol, pimozide, fluspirilene, trifluperidol, perazine, chlordiazepoxide, clobazam, diazepam, nordiazepam, flurazepam, lorazepam, nitrazepam, oxazepam, carbamazepine
**Interfering:** clozapine

### KEY WORDS
plasma; serum; column-switching

### REFERENCE
Härtter,S.; Wetzel,H.; Hiemke,C. Automated determination of fluvoxamine in plasma by column-switching high-performance liquid chromatography, *Clin.Chem.*, **1992**, *38*, 2082–2086.

### SAMPLE
**Matrix:** blood
**Sample preparation:** For each 1 mL plasma or serum add 10 μL 14 μg/mL trimipramine in MeOH. Inject serum or plasma directly onto column A with mobile phase A, elute with mobile phase A to waste. After 15 min elute column A onto column B (foreflush) with mobile phase B. After 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

### HPLC VARIABLES
**Column:** A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN
**Mobile phase:** A MeOH:water 5:95; B MeCN:MeOH:buffer 578:188:235 (Buffer was 10 mM $K_2HPO_4$ adjusted to pH 6.8 with 85% phosphoric acid.)

**Flow rate:** 1.5
**Injection volume:** 100
**Detector:** UV 214

**CHROMATOGRAM**
**Retention time:** 6.19
**Internal standard:** trimipramine (6.5)
**Limit of detection:** 1 ng/mL (with three injections onto column A before switching), 5-10 ng/mL

**OTHER SUBSTANCES**
**Extracted:** metabolites, amitriptyline, clomipramine, desipramine, doxepin, imipramine, maprotiline, nortriptyline
**Noninterfering:** chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozide, spiroperidol, trifluperidol

**KEY WORDS**
plasma; serum; column-switching

**REFERENCE**
Härtter,S.; Hiemke,C. Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum, *J.Chromatogr.*, **1992**, *578*, 273–282.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 1 mL Plasma + 10 µL 100 µg/mL nortriptyline in water + 100 µL 1 M pH 7.6 $K_2HPO_4$, vortex for 5 s, add 6 mL ethyl acetate, vortex for 1 min, centrifuge at 1900 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 µL 40 mM sodium bicarbonate, add 20 µL 10 mg/mL dansyl chloride in acetone, add 750 µL acetone, vortex for 30 s, let stand at room temperature at 22° for 15 min. Evaporate under a stream of nitrogen at 45°, reconstitute in 1 mL mobile phase, vortex for 1 min, centrifuge at 1900 g for 10 min, inject a 100 µL aliquot of the supernatant.

**HPLC VARIABLES**
**Guard column:** 15 × 3.2 7 µm NewGuard RP-8 (Brownlee)
**Column:** 250 × 4.6 5 µm Supelcosil LC-18-DB
**Mobile phase:** MeCN:10 mM pH 7.2 potassium phosphate 85:15
**Flow rate:** 1.5
**Injection volume:** 100
**Detector:** F (wavelengths not given)

**CHROMATOGRAM**
**Retention time:** 5.3
**Internal standard:** nortriptyline (9.7)
**Limit of quantitation:** 10 ng/mL

**OTHER SUBSTANCES**
**Simultaneous:** desipramine

**KEY WORDS**
plasma; derivatization

**REFERENCE**
Pullen,R.H.; Fatmi,A.A. Determination of fluvoxamine in human plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1992**, *574*, 101–107.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 µL 5 µg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 µL 100 mM HCl,

mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 μL 1 M pH 10.3 carbonate buffer and 25 μL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temperature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 μL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 μL aliquot.

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**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Supelcosil LC-18
**Mobile phase:** MeCN:25 mM $KH_2PO_4$ 75:25 + 500 μL/L orthophosphoric acid + 600 μL/L n-butylamine
**Flow rate:** 2
**Injection volume:** 25-40
**Detector:** F ex 235 em 470 (cut-off)

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**CHROMATOGRAM**
**Retention time:** 6.78
**Internal standard:** maprotiline (12.8)

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**OTHER SUBSTANCES**
**Simultaneous:** fluoxetine, propranolol, clovoxamine, fenfluramine, amoxapine, desipramine, protriptyline, nortriptyline, sertraline, norfluoxetine
**Noninterfering:** amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine

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**KEY WORDS**
plasma

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**REFERENCE**
Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

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**SAMPLE**
**Matrix:** blood
**Sample preparation:** Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 μL Serum + 50 μL 5 μg/mL trimipramine in 5% potassium bicarbonate + 700 μL MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL MeCN, elute with 250 μL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μL aliquot of the eluate.

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**HPLC VARIABLES**
**Guard column:** 15 mm 7 μm Brownlee RP-8
**Column:** 150 × 4.6 5 μm Ultrasphere Octyl
**Mobile phase:** MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
**Flow rate:** 1.5
**Injection volume:** 50
**Detector:** UV 215

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**CHROMATOGRAM**
**Retention time:** 6.8
**Internal standard:** trimipramine (9.6)
**Limit of quantitation:** 5 ng/mL

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**OTHER SUBSTANCES**
**Extracted:** amitriptyline, clomipramine, doxepin, fluoxetine, maprotiline, nortriptyline
**Interfering:** desmethylmaprotiline, desipramine, imipramine, protriptyline

**KEY WORDS**
serum; SPE

**REFERENCE**
Gupta,R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2751–2765.

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**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Serum + 5 mL 300 mM sodium phosphate + 400 μL diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!) + imipramine, mix for 20 min, centrifuge for 10 min, inject an aliquot of the organic layer.

**HPLC VARIABLES**
**Column:** 150 × 4.6 3 μm Apex Silica (Jones Chromatography)
**Mobile phase:** MeCN:MeOH:25% ammonia 345:65:1.7
**Flow rate:** 1.3
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 3.5
**Internal standard:** imipramine
**Limit of quantitation:** 0.5 nM

**KEY WORDS**
serum; normal phase; pharmacokinetics

**REFERENCE**
Spigset,O.; Carleborg,L.; Hedenmalm,K.; Dahlqvist,R. Effect of cigarette smoking on fluvoxamine pharmacokinetics in humans, *Clin.Pharmacol.Ther.*, **1995**, *58*, 399–403.

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**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

**HPLC VARIABLES**
**Column:** 300 × 3.9 4 μm NovaPack C18
**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
**Column temperature:** 30
**Flow rate:** 0.8
**Injection volume:** 50
**Detector:** UV 253

**CHROMATOGRAM**
**Retention time:** 8.71
**Limit of detection:** <120 ng/mL

**KEY WORDS**
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; car-

teolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

### REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

### SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Serum + oxprotiline, make alkaline with borate buffer, extract with cyclohexane:dichloromethane 60:40. Remove the organic layer and extract it with acid, inject an aliquot of the acid layer.

### HPLC VARIABLES

**Column:** C18 DB (Supelco)
**Mobile phase:** MeCN:pH 2.5 phosphate buffer 37:63
**Detector:** UV 254

### CHROMATOGRAM

**Internal standard:** oxprotiline

### OTHER SUBSTANCES

**Extracted:** bromazepam, clobazam, diazepam, lorazepam, oxazepam

### KEY WORDS

serum

### REFERENCE

Vandenberghe,H.; MacDonald,J.C. Analysis of fluvoxamine, clobazam and other benzodiazepines on the same HPLC system (Abstract 40), *Ther.Drug Monit.*, **1995**, *17*, 393–393.

### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the

organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 200.5

**CHROMATOGRAM**
**Retention time:** 15.347

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Pulverize a tablet, add 100 mL MeOH, shake mechanically for 5 min, centrifuge an aliquot at 3000 rpm for 5 min. Remove a 50 μL aliquot of the supernatant and add it to 50 μL 100 μg/mL propyl paraben in MeOH, make up to 1 mL with MeCN, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 100 × 8 10 μm μBondapack C18
**Mobile phase:** MeCN:50 mM ammonium acetate 60:40 adjusted to pH 5.2 with glacial acetic acid
**Flow rate:** 0.8
**Injection volume:** 20
**Detector:** UV 273

**CHROMATOGRAM**
**Retention time:** 9
**Internal standard:** propyl paraben (7)

**KEY WORDS**
tablets

**REFERENCE**
Foda,N.H. Quantitative analysis of fluvoxamine maleate in tablet formulations by HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 1591–1601.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
**Flow rate:** 0.6
**Injection volume:** 25
**Detector:** UV 229

## CHROMATOGRAM

**Retention time:** 9.97 (A), 5.94 (B)

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

## KEY WORDS

details of plasma extraction

## REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

## SAMPLE

**Matrix:** urine
**Sample preparation:** 8 mL Urine + 2 mL 100 mM pH 9.5 borate buffer, mix, filter (0.2 μm). Remove a 500 μL aliquot of the filtrate and add it to 100 μL 10 mM NaCN in 20 mM pH 9.5 borate buffer, add 500 μL 1 mM naphthalene-2,3-dicarboxaldehyde in MeOH, mix, let stand at room temperature for 20 min, add 100 μL 100 mM glycine in 20 mM pH 9.5 borate buffer, mix, let stand for 10 min, add 500 μL hexane:toluene 50:50, extract. Remove a 400 μL aliquot of the organic layer and add it to 800 μL dichloromethane, mix, inject a 35 μL aliquot.

## HPLC VARIABLES

**Column:** 250 × 3.1 5 μm LiChrosorb Si-60
**Mobile phase:** Dichloromethane:MeOH 99.8:0.2
**Flow rate:** 0.5
**Injection volume:** 35

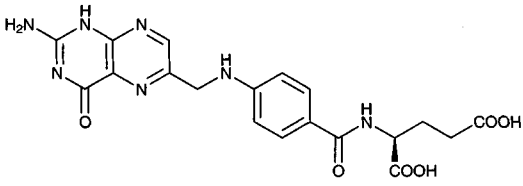
**Detector:** Chemiluminescence (418 nm cutoff filter) following post-column reaction. The column effluent mixed with 50 mM hydrogen peroxide in MeCN:dichloromethane 50:50 containing 0.5 mM triethylamine pumped at 0.1 mL/min and with 5 mM bis(2,4,6-trichlorophenyl) oxalate in dichloromethane pumped at 0.1 mL/min and the mixture flowed into the detector.

**CHROMATOGRAM**
**Retention time:** 7
**Limit of detection:** 5 nM

**KEY WORDS**
derivatization; normal phase

**REFERENCE**
Kwakman,P.J.M.; Koelewijn,H.; Kool,I.; Brinkman,U.A.T.; de Jong,G.J. Naphthalene- and anthracene-2,3-dialdehyde as precolumn labelling reagents for primary amines using reversed- and normal-phase liquid chromatography with peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1990**, *511*, 155–166.

# Folic acid



**Molecular formula:** $C_{19}H_{19}N_7O_6$
**Molecular weight:** 441.40
**CAS Registry No.:** 59-30-3
**Merck Index:** 4253

**SAMPLE**
**Matrix:** blood, formulations, urine
**Sample preparation:** Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4 5 μm Lichrosorb RP-18
**Mobile phase:** Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min
**Flow rate:** 1
**Injection volume:** 10
**Detector:** UV 270

**CHROMATOGRAM**
**Retention time:** 9.99
**Internal standard:** xanthine (4.65)
**Limit of detection:** 3 ng

**OTHER SUBSTANCES**
**Extracted:** ascorbic acid, niacin, niacinamide, riboflavin, vitamin B12

**KEY WORDS**
plasma; SPE; tablets; injections

**REFERENCE**
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.